ANALYTICAL DETERMINATION OF NICOTINE AND RELATED COMPOUNDS AND THEIR METABOLITES

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Chapter 16

Uses and abuses of cotinine as a marker of tobacco smoke exposure

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I. INTRODUCTION

Cotinine, as a biomarker of exposure to tobacco smoke, is typically measured in blood, saliva or urine. Cotinine levels in plasma and saliva are similar, but urinary levels are higher, generally by a factor of 5 to 6 [1]. Cotinine has also been detected in a wide range of other fluids, including amniotic fluid and breast milk [1], placental tissue [2], umbilical cord blood [3], semen [4], cerebrospinal fluid [5], cervical fluid [6] and even serum from a testicular biopsy [7]. Cotinine is also detectable in hair, though only at very low levels, and here nicotine is used as the marker of exposure [8].

There are seven main uses to which the measurement of cotinine has been put:

1. To provide evidence of exposure to tobacco smoke;
2. To compare exposure in smokers and non-smokers;
3. To use high levels in self-reported non-smokers to indicate that they are in fact true smokers;
4. To quantify the extent of exposure in cigarette smokers;
5. To compare exposure in different types of smokers;
6. To quantify the extent of ETS exposure in non-smokers;
7. To provide evidence of a relationship of tobacco smoke exposure with disease.

This chapter considers these uses in turn in Sections II to VIII, summarizing relevant results from the literature and commenting on the problems of interpretation that arise. Finally, some other issues relevant to the use of cotinine as a marker of tobacco smoke exposure are considered in Section IX and some general conclusions are reached in Section 1X.

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II. EVIDENCE OF EXPOSURE TO TOBACCO SMOKE

As will be seen in the following sections, there is no doubt that, on average, cotinine levels are substantially higher in smokers than in non-smokers, and are positively associated with the amount smoked by smokers and with the extent of ETS exposure in non-smokers. Although, in most cases, the presence of cotinine in body fluids arises as a result of active or passive smoking, it should be borne in mind that this need not necessarily be the case.

The most obvious alternative explanation for the presence of cotinine is exposure to other sources of nicotine, including use of smokeless tobacco products, chewing of nicotine gum, and use of nicotine patches or other aids for smoking cessation, and also occupational exposure to tobacco leaves or to nicotine products [1]. Chemical analytical error may, in theory, lead to a false reporting of the presence of cotinine, although this should not arise if appropriate methods are used (see Chapters 6, 7 and 8).

Dietary nicotine

Nicotine has been shown to be present in measurable quantities in several foods, including tomatoes, aubergines (egg plant) and other solanaceous vegetables, tea and potatoes [9–11], and there has been some discussion in the literature regarding the possible effect that dietary intake of nicotine might have on the use of cotinine as a marker of tobacco smoke exposure. Claims that dietary nicotine might be important are not well supported. One author [12] concluded that "a person who consumed 10 cups of tea in a day, ate food comprising 1 lb of tomatoes, aubergines or peppers, and 1 lb of potatoes with their skins, might ingest nicotine equivalent to 1–2 cigarettes", overestimated the nicotine equivalent by almost two orders of magnitude, due to a confusion between dry and wet weight in the calculations. Another author [13] presented calculations demonstrating that nicotine intake from someone eating a pound of potatoes is similar to that from someone spending about half a day in a smoky room with a low concentration of ETS, and concluded that dietary intake may be of practical importance when using cotinine levels to quantify the extent of ETS exposure in non-smokers. However the ETS concentration used in these calculations, of 1 μg nicotine per m^3, is really quite low, 5-10 $\mu g/m^3$ being common and 50 $\mu g/m^3$ being sometimes found in bars and nightclubs [14]. In fact, though nicotine from dietary sources is potentially detectable in cotinine assays, there is no indication that it would more than minimally interfere with the use of cotinine as a quantitative marker of ETS exposure. Most researchers have argued that diet is a relatively trivial source of nicotine [15–18] compared not only to smoking but also to moderate ETS exposure. This view is supported by results from two epidemiological studies which related cotinine

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levels to reported dietary intake. In one large US study [19] it was estimated that the contribution of diet to serum cotinine level was less than 0.020 ng/ml, more than 10 times less than that related to ETS exposure, and 1000 times or so less than that found in smokers. Another study, in Scotland [20], found that cotinine levels in non-smokers reflected far better the nicotine in inhaled ETS than they did the nicotine in tea.

III. RELATIVE EXPOSURE IN SMOKERS AND NON-SMOKERS

Cotinine levels in smokers and non-smokers

A number of studies have compared average cotinine levels in self-reported smokers and non-smokers. Tables 1 (urine), 2 (serum or plasma) and 3 (saliva) summarize the findings from some of the larger studies. The tables show the population studied, the chemical analytical technique used for cotinine determination, the type of average used (mean or median) and whether non-smokers with high cotinine levels have been omitted from the averages used.

In the serum, plasma or saliva of smokers, average cotinine levels are typically in the range 200–350 ng/ml. Exceptionally they are in the range 100–150 ng/ml in pregnant women or in adolescents. In non-smokers levels are typically no more than about 1 ng/ml, provided they are calculated as medians or as means excluding non-smokers with high cotinine levels typical of smokers. Means including such individuals, e.g. in the studies of Perez-Stable [32], Pojer [33], Richmond [35] and McNeill [39], tend to be higher, about 5 ng/ml. For studies of adults, and discounting means which include non-smokers with high cotinine levels, the ratio of average cotinine for smokers compared to non-smokers typically exceeds 200. This is evident not only from the three large representative studies conducted in the UK in 1993, 1994 and 1996 [27,28,34], but also from a large representative study undertaken in the US in 1988–1991 [19]. In that study, which used state-of-the-art high-performance liquid chromatography atmospheric-pressure chemical ionization tandem mass spectrometry (LC-MS/MS) [40], non-tobacco users aged 17+ had geometric mean cotinine levels of about 0.2 ng/ml, over 500 times lower than those seen in tobacco users, which appeared to be of order 300 ng/ml from a distribution given graphically.

For cotinine in urine, typical levels in the Jarvis [22], Thompson [25] and Wald [26] studies, whether in ng/ml or in ng/mg creatinine, were about 1500 in smokers and about five in non-smokers, leading to a ratio of about 300. Again, studies which did not exclude non-smokers with high cotinine levels [21,23,24] gave much lower ratios. Two of these studies [23,24] reported cotinine levels that were exceptionally high, leading to doubts about the methodology used [41].

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TABLE 1

Cotinine levels in urine (ng/ml or ng/mg creatinine)

Study	Population	Method[1]	Units	Type of average[2]	N[3]	Cotinine level[4]		
						Non-smoker	Smoker	Ratio
Galanti [21]	Belgium/adults aged 18–29	FPI/Cr	ng/mg	Mean	2431	32.0	717	22.4
Jarvis [22]	UK/adult outpatients	GC	ng/ml	Mean*	190	4.8	1404	293
Mathai [23]	UK/pregnant females	SP/Cr	ng/mg	Mean	285	452	5980	13.2
Matsukura [24]	Japan/adults	RIA/Cr	ng/mg	Mean	744	680	8570	12.6
Thompson [25]	UK/adult males	RIA/Cr	ng/mg	Median	233	6.1	1623	266
		RIA/Cr	ng/mg	Mean	233	13.4	1691	126
Wald [26]	UK/adult males	RIA	ng/ml	Median	453	4.0	1474	369

[1] Method: GC = gas chromatography, SP = spectrophotometric, RIA = radioimmunoassay, FPI = fluorescence polarization immunoassay, /Cr = corrected for creatinine.

[2] Type of average: * indicates that non-smokers with cotinine levels typical of smokers have been excluded from the calculation of the mean.

[3] N: number of non-smokers and smokers combined.

[4] Cotinine level: where appropriate, levels have been estimated from data by level of ETS exposure or by smoking category, or converted from nmol/ml to ng/ml.

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TABLE 2

Cotinine levels in serum or plasma (ng/ml)

Study	Population	Method[1]	Type of average[2]	N[3]	Cotinine level[4]		
					Non-smoker	Smoker	Ratio
Bennett [27]	UK/adults	GC	Mean*	4864	1.1	217	198
Colhoun [28]	UK/adult males	GC	Mean*	4772	0.9	232	255
	UK/adult females	GC	Mean*	5242	0.8	230	299
Haddow [29]	US/adult females	RIA	Mean	296	2.9	304	105
			Median	296	1.1	305	277
Haddow [30]	US/pregnant females	RIA	Median	4211	<10	134	?
Jarvis [22]	UK/adult outpatients	GC	Mean*	190	1.5	280	187
Kemmeren [31]	Holland/adult males	GC	Mean	253	1.1	327	304
Perez-Stable [32]	US/Mexican adults	GC	Mean	743	5.3	170	32.2
Pojer [33]	Australia/adults[5]	GC	Mean	368	4.4	335	76.2
Prescott-Clarke [34]	UK/adult males	GC	Mean*	5446	1.0	235	233
	UK/adult females	GC	Mean*	5996	0.8	222	266
Richmond [35]	Australia/adults[6]	GC	Mean	420	5.0	335	67.0
Tunstall-Pedoe [20]	UK/adult males	GC	Median	3813	0.7	250	368
	UK/adult females	GC	Median	3657	0.1	243	2430
Wald [36]	UK/adult males	RIA	Mean	304	0	276	∞

[1] Method: GC=gas chromatography, RIA=radioimmunoassay.
[2] Type of average: * indicates that non-smokers with cotinine levels typical of smokers have been excluded from the calculation of the mean.
[3] N: number of non-smokers and smokers combined.
[4] Cotinine level: where appropriate levels have been estimated from data by level of ETS exposure or by smoking category, or converted from nmol/ml to ng/ml.
[5] In smoking cessation campaign or attending clinic.
[6] Smokers in smoking cessation program.

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TABLE 3

Cotinine levels in saliva (ng/ml)

Study	Population	Method[1]	Type of average[2]	N[3]	Cotinine level[4]		
					Non-smoker	Smoker	Ratio
Jarvis [22]	UK/adult outpatients	GC	Mean*	190	1.7	314	185
Jarvis [37]	UK/children aged 16–19	GC	Median	699	0.6	130	217
Lee [17]	UK/adult females	GC	Median	422	0.9	311	365
	UK/adult males	GC	Median	562	0.4	319	798
Li [38]	US/pregnant females	RIA	Mean*	1279	1.1	123	112
McNeill [39]	UK/girls aged 11–16	GC	Mean	508	4.6	123	27

[1] Method: GC=gas chromatography, RIA=radioimmunoassay.

[2] Type of average: * indicates that non-smokers with cotinine levels typical of smokers have been excluded from the calculation of the mean.

[3] N: number of non-smokers and smokers combined.

[4] Cotinine level: where appropriate levels have been estimated from data by level of ETS exposure or by smoking category, or converted from nmol/ml to ng/ml.

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Factors affecting estimates of the ratio of cotinine levels in smokers and non-smokers

There are a number of issues that are relevant to the ratio of cotinine levels in smokers and non-smokers. One problem, as already noted, is that the ratio may be substantially underestimated if some smokers misreport their smoking and have their high cotinine levels included in the average for non-smokers. While this has little effect on the median, it biases the mean upward, sometimes substantially. Even one smoker with a serum cotinine of 300 ng/ml can double the estimate of the mean, when erroneously included within a sample of 300 non-smokers, with a mean cotinine of 1 ng/ml.

Another problem relates to analytical accuracy at low levels of cotinine. The lower the level of cotinine, the higher the percentage variability, and all methods, with the possible exception of LC-MS/MS [40], have a minimum detection limit that is higher than the level seen in a proportion of non-smokers. For values below the detection limit, there is uncertainty as to what value should be included in the calculation of the mean, and for values just above it there is considerable doubt about its reliability. While one can have moderate confidence about the reliability of the average level in smokers reported in recent studies using appropriate methods, the average level reported in non-smokers, and hence the calculated ratio of levels, is far less precise.

The effect the choice of analytical method has on the ratio of levels is well illustrated by a study [42] in which 11 laboratories from 6 countries, all experienced in performing cotinine determinations in biological fluids by radioimmunoassay and/or gas chromatography, each received 18 serum and 18 urine samples. 8 from smokers and 10 from non-smokers (2 spiked with defined amounts of nicotine and cotinine). While all the laboratories distinguished perfectly between the smokers and non-smokers, and the ranking of the samples agreed well over laboratory, there were systematic differences in absolute values between the laboratories. Notably, the ratios of urinary cotinine concentrations between active and passive smokers differed widely from laboratory to laboratory, ranging from 21 to 294.

As noted in the previous section, dietary intake of nicotine is only likely to have a small effect on cotinine levels. As its contribution will be trivial in smokers, but may be less so in non-smokers, the effect of ignoring diet will be to underestimate somewhat the ratio of cotinine levels in smokers compared to non-smokers.

Interpretation of the ratio of cotinine levels in smokers and non-smokers

Apart from the technical problems in obtaining an accurate estimate of the ratio of cotinine levels in smokers compared to non-smokers, one must bear

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in mind the extent to which such a ratio can be interpreted as an index of relative exposure to tobacco smoke constituents. In the first place, cotinine is at best only a marker of recent exposure to nicotine, and is not necessarily a marker of lifetime exposure of, or exposure to, other tobacco smoke constituents. Nicotine is predominantly in the particulate phase of mainstream smoke and in the vapour phase of ETS [43] and the pattern of absorption, which depends on the pH of the smoke [14], will differ for smokers and non-smokers. It has been estimated [43] that the dose ratio between smoking and ETS exposure, while about 100–125 for nicotine, is much higher for particles, (1700–4000), and for tobacco specific nitrosamines, (3000–6000), and much lower for vapour phase constituents such as carbon monoxide, (2.7–4.2) or volatile nitrosamines, (1.5–2.5).

Even as a marker of exposure to nicotine, cotinine is only one of a number of metabolites and smokers and non-smokers may differ in their patterns of metabolism, as discussed further below.

For these reasons, one certainly cannot assume that, just because average cotinine levels in smokers may be 300 times, say, higher than those in non-smokers, it necessarily follows that exposure to those tobacco smoke constituents that are relevant to a specific disease is 300 times greater in smokers than non-smokers [44,45].

IV. RECLASSIFYING SELF-REPORTED NON-SMOKERS AS SMOKERS

If some smokers fail to report they smoke, the prevalence of smoking in the population concerned will be underestimated. When attempting to study the relationship of ETS exposure to risk of lung cancer (or other diseases strongly related to smoking) among non-smokers, the inclusion of a small proportion of misclassified smokers in the study population can cause important bias, and create an apparent relationship of ETS exposure to risk even when no true relationship exists [46].

Using cotinine levels to estimate misclassification rates

Failure to report previous smoking can be demonstrated from studies which have recorded smoking habits in the same individuals at different points in time [47]. Failure to report current smoking can be detected using cotinine. The rationale for regarding high cotinine levels as indicative of current smoking, rather than of extreme ETS exposure, is based on two main lines of argument. First, the distribution of cotinine values in those not reporting smoking but reporting ETS exposure is usually found to take the form of a continuous log-normal distribution with a mean a 100 times or more lower than that of smokers, together with a few very high values that do not fit the distribution at all, but are typical levels for smokers [17,19]. Second, non-

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smokers exposed to maximally tolerable levels of ETS in experimental chamber studies [48,49] or working in occupations with very high ETS exposure [50,51] are not found to have cotinine levels that even approach typical levels seen in smokers. The EPA has suggested that self-reported non-smokers with cotinine levels above 10% of smokers' levels can definitely be regarded as true smokers, and that cotinine levels above 30% should be regarded as indicating regular smoking [52]. However, a number of researchers use rather lower levels, e.g. 8 to 20 ng/ml in serum or saliva [47], to reclassify non-smokers as smokers. At the end of the day, cotinine levels cannot completely distinguish between non-smokers with high ETS exposure and very light active smokers. However, they can indicate levels that are certainly not due to ETS and, provided a satisfactory attempt is made to exclude other sources of nicotine such as gum or patches, are due to active smoking.

Levels of misclassification

Table 4 summarizes evidence on misclassification rates from over 40 studies. Three types of rate are shown:

A. percentage of self-reported non-smokers found to be smokers.
B. percentage of true current smokers (i.e. including misclassified smokers) who deny current smoking and report being non-smokers.
C. percentage of true current smokers who deny current smoking and report never having smoked.

Rate A can be calculated for all the studies (and the studies are presented in ascending order of this), but, as it depends on the underlying frequency of smokers in the population, is less valuable than rates B or C. Rate B is only available if the study was not restricted to self-reported non-smokers, while rate C additionally requires data to have been collected on past smoking.

Factors affecting estimated misclassification rates

As is evident from Table 4, misclassification rates vary widely between studies. There are a number of reasons for this. These include the precise choice of cut-off used, the methodology used for cotinine determination, the underlying prevalence of smoking in the population (which should only affect rate A) and failure in some studies to ask questions about all types of smoking or to consider other sources of nicotine (though many studies were very thorough in this respect). A particularly important reason is that study populations vary in willingness to admit smoking when asked.

A striking observation from Table 4 is that, in the three studies of Asian women, two conducted in Japan [74,75] and one in South-East Asian women living in the USA [76], the proportion of true smokers denying smoking (rate B) is consistently high (26.4%, 40.7% and 64.6%). Also, unlike in Western

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TABLE 4

Rates of misclassification of current smoking[1]

Study	Country[2]	Sex	Body fluid	Cut-off	Current smokers among self-reported non-smokers: n	% (A)	% denying current smoking and reporting as: Non-smokers (B)	Never smokers (C)
Emmons [53]	USA	M+F	Saliva	10 ng/ml	1	0.5		
Cummings [54]	USA	M+F	Urine	90 ng/ml	6	0.9		
Wald [26]	UK	M+F	Urine	128 ng/ml	2	0.9	0.9	
Delfino [55]	Canada	M+F	Saliva	20 ng/ml	3	1.2		
Pirkle [19]	USA	M+F	Serum	15 ng/ml	78	1.3	2.0	
Martinez [56]	USA	F	Serum	20 ng/ml	2	1.4	5.1	
Thompson [25]	UK	M+F	Urine	100 ng/mg	3	1.6	5.8	
English [57]	USA	F	Serum	14 ng/ml	42	1.7	3.4	2.3
Haley [58]	USA	M+F	Urine	100 ng/mg	5	1.7		
Haddow [59]	USA	F	Serum	10 ng/ml	29	1.9		
Riboli [60]	Many	F	Urine	100 ng/mg	26	1.9		
Slattery II [61][3]	USA	F	Serum	15 ng/ml	8	2.1	4.7	2.3
Haddow [29]	USA	F	Serum	16 ng/ml	5	2.2	7.2	
Fontham [62]	USA	F	Urine	100 ng/mg	25	2.3		
Lee [17]	UK	M+F	Saliva	30 ng/ml	20	2.5	3.1	
Heller II [63][3]	Germany	M+F	Serum	15 ng/ml	65	2.8	5.7	1.2
Dickinson [64]	Australia	M+F	Saliva	35 ng/ml	10	2.8	7.7	
Heller A [63][3]	Germany	M+F	Serum	15 ng/ml	72	3.0	5.5	0.8
Colhoun [28]	UK	M+F	Serum	20 ng/ml	232	3.2	7.0	1.8
Heller B [63][3]	Germany	M+F	Serum	15 ng/ml	71	3.2	7.6	1.8
Tunstall-Pedoe [20]	UK	M+F	Serum	20 ng/ml	141	3.4	4.1	
Bennett [27]	UK	M+F	Serum	20 ng/ml	121	3.5	7.3	2.8
Luepker [65]	USA	M+F	Saliva	20 ng/ml	8	3.6	6.5	4.1
Becher [66]	Germany	F	Urine	100 ng/ml	7	3.6		
Van Vunakis [67]	USA	M+F	Saliva	25 ng/ml	7	3.8	5.6	

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TABLE 4 Continued

Study	Country[2]	Sex	Body fluid	Cut-off	Current smokers among self-reported non-smokers: n	% (A)	% denying current smoking and reporting as: Non-smokers (B)	Never smokers (C)
Haddow [30]	USA	F	Serum	25 ng/ml	112	3.9	7.7	
Wagenknecht [68]	USA	M+F	Serum	14 ng/ml	145	4.2	8.6	4.3
Pierce [69]	Australia	M+F	Saliva	34 ng/ml	31	5.0	8.1	
Ogden [70]	USA	F	Saliva	10%[5]	45	5.5	14.1	6.4
Coultas [71]	USA	M+F	Saliva	20 ng/ml	51	5.7	14.6	7.1
Van Vunakis [67]	USA	M+F	Serum	8 ng/ml	11	5.7	8.0	
Perez-Stable [32]	USA	M+F	Plasma	14 ng/ml	12	6.3	11.4	4.8
Slattery I [61][3]	USA	M	Serum	15 ng/ml	7	6.4		
Ford [72]	N.Z.	M	Serum	15 ng/ml	30	6.6	26.1	
Ogden [73]	USA	F	Saliva	10 ng/ml	8	7.7		
Lee [74]	Japan	F	Urine	100 ng/mg	28	8.8	26.4	20.8
Akiyama [75]	Japan	F	Urine	100 ng/mg	11	9.2	40.7	36.0
Wewers [76]	Asia[6]	M	Saliva	14 ng/ml	43	9.3	11.8	6.0
Wewers [76]	Asia[6]	F	Saliva	14 ng/ml	64	10.9	64.6	61.6
Luepker [65]	USA	M+F	Saliva	20 ng/ml	31	12.4	26.3	5.9
Phillips [77]	UK	F	Saliva	25 ng/ml	41	12.5		
Slattery III [61][3]	USA	M	Serum	15 ng/ml	27	17.2		
Jarvis [22]	UK	M+F	Plasma	14 ng/ml	21	17.4	18.9	

[1] Adapted from Lee [47], with data from additional studies added.
[2] N.Z. = New Zealand.
[3] Results were reported for three different study populations; see [47].
[4] Heller A and Heller B are results from the same sample at two time points three years apart, Heller II are results from a second sample.
[5] 10% of smokers' levels.
[6] Study conducted in US but restricted to subjects from Laos, Cambodia or Vietnam.

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populations, where most misclassified current smokers admit past smoking and have average cotinine levels that are substantially lower than in those who admit their current smoking, the Asian women identified as misclassified current smokers predominantly claim never to have smoked and to have cotinine levels typical of current smokers who admit it. The high misclassification rates in Asian women no doubt occur because smoking by women is socially unacceptable there.

It is well documented [78–80] that misclassification rates are likely to be particularly high in studies where patients are advised to give up smoking, patients being unwilling to admit to their doctor or to their spouse that they have failed to do what they were asked. Studies conducted in a situation where subjects had, or were likely to have had, advice to give up smoking and which reported high misclassification rates, include those by Jarvis et al. [22], Slattery et al. [61] and Ford et al. [72]. Higher than average misclassification rates were also noted in studies where non-smokers were paid to participate [77,81], concealment of smoking probably occurring for financial reasons. By contrast, screening studies, where subjects may be more ready to admit smoking, tended to produce quite low detection rates. These are only some of the factors affecting the extent to which smokers admit their habit. A comprehensive review of the evidence on misclassification of smoking as determined by cotinine gives a fuller discussion [47].

V. EXTENT OF EXPOSURE IN CIGARETTE SMOKERS

Cotinine is often used in an attempt to quantify the extent of exposure in cigarette smokers. Table 5 summarizes evidence from various studies of smokers that have reported the correlation between cotinine level and the reported number of cigarettes per day smoked, with results shown separately for urine, serum or plasma, and saliva. Although the studies are consistent in showing a positive correlation regardless of body fluid, the magnitude of the correlation is not very strong, typically in the range 0.35–0.50, though some studies report lower values.

A positive relationship is also evident from the data shown in Table 6, showing how cotinine level varies by grouped amount smoked, with results presented from three large representative surveys of the UK population [27,28,34], a population study in Germany [93], five studies in the US – two of pregnant women [30,57] and two not [37,95,96] – and two studies in Japan [24,74]. In virtually all these studies cotinine levels increase monotonically with increasing number of cigarettes smoked.

Do heavy smokers inhale less?

Although the relationship is monotonic, it may not be linear and there is a suggestion from some of the studies (though not all) that the increase in cotinine per cigarette becomes smaller with increasing number of cigarettes.

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TABLE 5

Correlation of cotinine level with number of cigarettes per day smoked (by cigarette smokers)

Body fluid	Study	Country	Sex	Sample size	Correlation coefficient
Urine	Bodmer [82]	UK	M+F	38	0.50
	Matsukura [24]	Japan	M+F	247	0.15
Serum/	Adlkofer [83]	Germany	M	200	0.41-0.44*
Plasma	Bennett [27]	UK	M+F	1290	0.49
	Klebanoff [84]	USA	F	192	0.44
	Kemmeren [31]	Holland	M	148	0.44
	Puntoni [85]	Italy	M+F	120	0.40
	Suadicani [86]	Denmark	M	1710	0.27
	Woodward [87]	UK	F	1939	0.46
			M	1574	0.49
	Worrell [88]	UK	F	240	0.38
			M	456	0.38
Saliva	Coultas [90]	USA	M+F	260	0.52
	Istvan [91]	USA	F	2096	0.15
			M	3538	0.21
	Pierce [69]	Australia	M+F	353	0.33
	Stanton [92]	New Zealand	M+F	265	0.38

* Cotinine samples taken at three time points.

The tendency for the slope to decrease as the number of cigarettes increases is more clearly seen in the final study [97], which subdivides cigarette consumption more finely. The results of the study are claimed by the authors to explain the anomaly that "the relationship between the number of cigarettes smoked per day and the incidence of lung cancer is linear, but, from the multistage model of carcinogenesis, it should be quadratic (upwards curving)." The authors effectively argue that use of number smoked per day as an index of exposure is misleading as heavier smokers inhale less smoke from each cigarette, and that cotinine is a better and, indeed, appropriate index of exposure.

While there is evidence that cotinine levels are higher in smokers who report that they inhale than in those who report that they do not [86,88], and while it may also be true that heavy smokers do inhale less than do light smokers, the argument is not as straightforward as the authors would have it. In the first place the machine nicotine yields of cigarettes smoked by heavy and light smokers may differ. Furthermore, heavy and light smokers may differ in exposure to other lung carcinogens. A non-linear relationship of cotinine to amount smoked may arise because metabolism of nicotine to cotinine is not a simple linear function of the dose of nicotine absorbed. It is also possible that differences between heavier and lighter smokers in relation to other factors (e.g. alcohol consumption, see Section 8) might affect metabolism. The fact that the relationship between cigarette consumption

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TABLE 6

Cotinine level in cigarette smokers by level of consumption

Study (Country)	Body fluid	Sex	Sample Size	Cotinine level (ng/ml unless stated) with number per day in brackets below				
Bennett [27]	Serum	M+F	1270	115	248	319		
(UK)				(1-9)	(10–19)	(30+)		
Colhoun [28]	Serum	M	1350	151	269	323		
(UK)		F	1365	118	253	306		
				(1-9)	(10–19)	(20+)		
Prescott-Clarke [34]	Serum	M	1638	144	270	333		
		F	1592	110	246	308		
(UK)				(1-9)	(10–19)	(20+)		
Heller [93]	Serum	M	502	49	133	209	278	348
(Germany)		F	385	46	113	212	278	355
				(Occ.)	(1-9)	(10–19)	(20–29)	(30+)
English [57]	Serum	F**	374 Blacks	91	149	182		
(USA)			829 Whites	44	128	164		
				(1-9)	(10–19)	(20+)		
Haddow [30]	Serum	F**	1340	68	119	135	159	184
(USA)				(1-7)	(8–12)	(13–17)	(18–24)	(25+)
Perez-Stable [94]	Serum	M	287	70	181	243		
(USA)		F	260	84	160	257		
				(1-9)	(10–19)	(20+)		
Sepkovic [95]	Plasma	M	958	141	261	332	375	381
(USA)		F	749	125	363	315	322	404
				(1–10)	(11–20)	(21–30)	(31–40)	(41+)
Ahijevych [96]	Saliva	F	142	360	399	407		
(USA)				(109)	(10–19)	(20+)		
Lee [74]	Urine*	F	78	259	1018	1433	2647	
(Japan)				(1–5)	(6–10)	(11–15)	(16+)	
Matsukura [24]	Urine*	M+F	247	310	420	870	1030	1560
(Japan)				(1-9)	(10–19)	(20–29)	(30–39)	(40–49)
Law (97)	Serum	M	1175*	45	130	247	249	262
(UK)				(1-9)	(10–14)	(15–19)	(20)	(20–24)
				301	287	338	343	
				(25–29)	(30–34)	(35–39)	(40+)	

* ng/mg creatinine. ** pregnant women * includes never smokers.

and risk of lung cancer is less steep than predicted by the multistage model might also be due to inadequacies in this model and to random errors in reported cigarette consumption, which would tend to flatten out the observed slope.

In any case, the results of this study [97] seem inconsistent with that of another study [98] of 116 smokers, in which saliva cotinine was measured and daily cigarette consumption logged into a hand-held computer. That study reported a non-linear relationship in which cotinine levels rose rapidly

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up to 10 cigarettes/day, then flattened off, and then rose again above 20 cigarettes/day. In that study, saliva cotinine was also noted to be positively associated with age.

Variation in inhalation and metabolism by amount smoked and errors in reporting amount smoked may all contribute to the relationship between cotinine and daily number of cigarettes smoked not being a strong one. The correlation may also be weakened because the daily number of cigarettes smoked on average may not correspond to the actual number smoked during the days to which the cotinine value was relevant and, probably more importantly because of the considerable inter-individual variation in cotinine levels between smokers of the same number of cigarettes/day. This may arise due to differences in the cigarettes smoked, inhalation, absorption and metabolism.

VI. COMPARING EXPOSURE FOR DIFFERENT TYPES OF SMOKERS

Pipe and cigar smokers

Table 7 compares cotinine levels in pipe and cigar smokers with levels in cigarette smokers based on data from three large representative studies of the UK [27,28,34], five other UK studies [17,22,26,36,87] and one study in Germany [93]. In the German study, cotinine levels in both pipe-only smokers and cigar-only smokers are lower than in cigarette smokers. In the UK studies, however, the results are consistent in showing that while cigar smokers have a markedly lower cotinine level than cigarette smokers, pipe smokers have a similar level. The similarity of cotinine levels in pipe and cigarette smokers, coupled with evidence that heart disease rates are substantially higher in cigarette than in pipe smokers, was used by Wald et al. [36] to infer that nicotine is not the cause of coronary heart disease. This inference is, however, open to question. The pattern of exposure to nicotine in cigarette smokers (intermittent series of high-nicotine boli) differs sharply from that in pipe smokers (slower absorption without peaks) [99], and similar levels of cotinine need not necessarily imply similar levels of circulating nicotine [100].

Table 7 also shows that cotinine levels in smokers of cigars only or in smokers of pipes or cigars are consistently higher in those who have smoked cigarettes in the past than in those who have not done so. This is consistent with inhalation in smokers of cigars only being shallower than in smokers of cigarettes only, but with cigarette smokers switching to cigars tending to retain their inhaling habits.

Handrolled cigarette smokers

Few studies have compared cotinine levels in smokers of handrolled cigarettes and smokers of manufactured cigarettes. Those that have done so suggest rather higher levels in handrolled cigarette smokers [17,93].

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TABLE 7

Cotinine levels in smokers of pipes and cigars[1]

Study	Country	Body fluid	Product smoked	Cigarette smoking	Sample size	Cotinine level (ng/ml)	Level in cigarette only smokers
Bennett [27]	UK	Serum	Pipe or cigar	Ex	118	151	234
		Serum	Pipe or cigar	Never	71	49	
Colhoun [28]	UK	Serum	Pipe or cigar	Ex	227	136	263
		Serum	Pipe or cigar	Never	112	57	
Prescott-Clarke [34]	UK	Serum	Pipe or cigar	Ex	226	150	263
		Serum	Pipe or cigar	Never	147	56	
Lee [17]	UK	Saliva	Cigar only	Non	9	10	310
Jarvis [22]	UK	Plasma	Pipe or cigar	Non	15	210	294
		Saliva	Pipe or cigar	Non	15	231	330
		Urine	Pipe or cigar	Non	15	1185	1448
Wald [26]	UK	Urine	Cigar only	Non	59	396	1645
		Urine	Pipe only	Non	42	1920	
Wald [36]	UK	Serum	Cigar only	Ex	55	149	306
		Serum	Cigar only	Never	15	18	
		Serum	Pipe only	Ex	33	415	
		Serum	Pipe only	Never	23	353	
Woodward [87]	UK	Serum	Cigar only	Non	319	9	283
		Serum	Pipe only	Non	39	259	
		Serum	Pipes and cigars	Non	83	203	
Heller [93]	Germany	Serum	Pipe only	Non	153	92	233
		Serum	Cigar only	Non	136	79	

[1] Data shown are for males or predominantly for males.

Compensation by smokers of lower tar and nicotine cigarettes

Many experimental and observational studies have suggested that smokers who switch to a brand with a lower tar and nicotine level, as measured by a smoking machine under standard conditions, adjust their inhalation pattern in order to compensate [101]. A review of the evidence using three commonly used markers of intake, carboxyhaemoglobin, breath carbon monoxide and blood nicotine, suggests that though cigarette smokers switching to a lower tar and nicotine brand do compensate to some extent, such compensation is not complete, so they do inhale less carbon monoxide and nicotine [102]. More recent compensation studies have used cotinine as the marker, which seems logical if, as is commonly thought [103], nicotine determines the extent of smoke intake, given that cotinine is the major metabolite of nicotine.

Some investigators have attempted to quantify compensation by means of an index α, which relates cotinine level C to brand nicotine yield Y by the model $C=\mu Y^{1-\alpha}$. Here $\alpha=1$ implies complete compensation, with cotinine

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level independent of nicotine yield, and $\alpha=0$ implies no compensation, with cotinine level directly proportional to nicotine yield. In one such study [83], of 103 German cigarette smokers supplying blood samples on six occasions, α was estimated as 0.755, after adjustment for amount smoked. This is equivalent to a 50% reduction in brand nicotine yield leading to a 16% reduction in cotinine level. Similar conclusions were reached in a UK intervention trial [104] in which over 400 subjects were randomly allocated to change rapidly or slowly to a brand with a tar yield of about half that of their usual brand. Data collected at five time points included serum cotinine and, after adjustment for amount smoked, the authors estimated an average compensation index for nicotine of 0.79, with the 50% reduction in yield predicted to result in an estimated 14% reduction in cotinine.

In a later study in Germany [93], in which serum cotinine was determined in a representative study of over 4000 subjects, the compensation index was estimated as 0.70 (or a 19% reduction in cotinine for a 50% reduction in yield). Unlike the previous two studies, which looked at within-smoker changes, this looked at between-smoker changes. Such estimates may be biased downwards if more 'dedicated' smokers, who would inhale more than average under comparable circumstances, tend to choose to smoke higher yield cigarettes. In this context, it should be noted that the first German study [83] also presented compensation indices based on between-smoker differences which, averaging 0.57, were lower than the estimated value within-smoker. Though some studies, e.g. [88], have reported no significant association between cotinine level and brand nicotine yield, the literature generally suggests that though smokers compensate, they do so partly but not completely. While the evidence summarized here does not demonstrate directly that smokers compensate for nicotine specifically, and it has been suggested that titration for tar may occur [89], it is of interest to note that the UK intervention trial cited above (104) reported lower compensation indices for carbon monoxide (0.65) and for tar (0.62) than it did for nicotine (0.79).

VII. EXTENT OF ETS EXPOSURE IN NON-SMOKERS

Evidence that ETS exposure might increase the risk of lung cancer in non-smokers was first derived from two studies conducted in 1981 [105,106] which reported that non-smoking women married to smokers had a higher risk than did non-smoking women married to non-smokers. Since then many other studies have used smoking by the husband as an index of ETS exposure in non-smoking women.

Cotinine levels and smoking by the husband

Table 8 compares cotinine levels in non-smoking women according to their husband's smoking habits (or in some cases according to their partner's

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TABLE 8

Cotinine levels in non-smokers[1] in relation to smoking by the husband

Study	Country	Body fluid	Sample size	Cotinine level (ng/ml)[2]		
				Husband non-smoker	Husband smoker	Ratio (Z)
Becher [66]	Germany	Urine*	93	5.5	18.0	3.27
	Poland	Urine*	90	6.1	16.3	2.68
Cummings [54]	USA	Urine	225	6.8	10.5	1.55
Heller [63][3]	Germany	Serum	1936	0.36	0.95	2.66
Lee [17]	UK	Saliva	72	0.3	1.0	3.33
Lee [74]	Japan	Urine	264	18.0	11.5	0.64
Ogden [73]	USA	Urine	96	0.27	2.3	8.63
Phillips [77][4]	UK	Saliva	106	0.25	1.3	5.20
	UK	Saliva	101	0.25	0.90	3.60
Pirkle [19][5]	USA	Serum	2672	0.20	0.77	3.85
Rebagliato [107]	Spain	Saliva	698	0.73	1.28	1.75
Riboli [60][5]	Many	Urine*	1322	3.23	9.26	2.87
Wald [108]	USA	Urine	121	8.5	25.2	2.96
Willers [109]	Sweden	Urine	34	1.4	6.6	4.71

[1] Self-reported non-smokers with high cotinine levels indicative of smoking have generally been removed from the analyses presented.
[2] ng/mg creatinine where indicated by an asterisk in the body fluid column.
[3] Data are for partner smoked and are for sexes combined.
[4] Results are for pre-monitoring (line 1) and post-monitoring (line 2) cotinine levels, and are for smoking by the partner.
[5] Data are for any household exposure.

habits or to smoking in the household). It can be seen that, with the striking exception of the single study in Japan [74], where cotinine levels were non-significantly lower if the husband smoked, the studies consistently show higher cotinine levels in the partner if the husband smoked.

Z ratios

A number of meta-analyses of the evidence relating husband's smoking to lung cancer, (e.g. [14,52,110,111]), have attempted to correct overall estimates of relative risk for the background ETS exposure of wives of non-smoking husbands. The method used assumes that the relationship between risk of lung cancer and ETS exposure is linear, and that the ratio of ETS exposure for women married to a smoker and for women married to a non-smoker is validly estimated by their relative cotinine exposure Z. Given these assumptions, the meta-analysis risk estimates for women married to a smoker, relative to women married to a non-smoker (R) can be converted into an estimate, relative to totally unexposed women, (R'), by using the formula

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$$R' = 1 + Z(R-1)/(Z-R)$$

while the risk for women married to a non-smoker relative to totally unexposed women, R'', can be estimated using the formula

$$R'' = 1 + (R-1)/(Z-R).$$

For example, if $Z=3$ and $R=1.25$, the relative risk estimates adjusted for background become $R'=1.43$ (married to a smoker) and $R''=1.14$ (married to a non-smoker).

While it is reasonable to assume that the higher cotinine levels seen in non-smoking women married to a smoker indicate they receive greater ETS exposure, there are a number of problems with this background adjustment procedure [112]. In the first place, as can be seen from Table 8, there is considerable variability between studies in the estimated Z ratio. Thus, while one review [110] used the value of three obtained from an early UK study [108], another review [52] used a much lower value of 1.55, based on a single US study [54]. For the same R value of 1.25, the differing Z values have a large effect on the estimates of R' (1.43 for $Z=3$, 2.29 for $Z=1.55$), with a consequently large effect on the estimated risk of lung cancer attributable to ETS.

Variations between studies in the estimated Z ratio may arise for a number of reasons. These include sampling error, analytical error at low cotinine levels (which will depend on the method used) and differences between the study populations in the extent to which men smoke and to which husbands smoke in the presence of the wife. The differing relative importance, in the differing study populations, of other sources of nicotine exposure (including other sources of ETS exposure, nicotine replacement products and diet) would also affect the Z ratio. Though marriage to a smoker may be a reasonable marker of increased exposure one must have distinct reservations about the described use of Z ratios, particularly when the populations investigated in the lung cancer studies differ from those used to estimate Z.

Cotinine levels and workplace ETS exposure

Table 9 shows that cotinine levels in non-smokers are also increased in relation to workplace ETS exposure. However, exposed/unexposed ratios tend to be lower than for spousal smoking (Table 8) suggesting that, on average, marriage to a smoker is a more important source of ETS exposure than is working with a smoker. This conclusion is consistent with that of a number of studies which show higher cotinine levels in those with ETS exposure only at home than in those exposed only at work. For example, the IARC multicountry study [60] reported urinary cotinine levels of, respectively, 2.7, 4.8, 9.0 and 10.0 ng/mg creatinine for non-smoking women with reported ETS exposure neither at home nor at work, at work only, at home only, and both at home and at work. Similarly, in a large US study of both

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TABLE 9

Cotinine levels in non-smokers[1] in relation to workplace ETS exposure

Study	Country	Body fluid	Sample size	Cotinine level (ng/ml)[2]		
				Workplace ETS exposure		
				No	Yes	Ratio
Becher [66]	Germany	Urine*	93	8.6	8.8	1.03
	Poland	Urine*	90	8.1	13.4	1.65
Matsukura [24]	Japan	Urine*	277	0.22	0.72	3.27
Ogden [73]	USA	Urine	96	0.64	0.93	1.45
Pirkle [19]	USA	Serum	2672	0.23	0.46	2.00
Rebagliato [107]	Spain	Saliva	698	0.85	1.3	1.53
Riboli [60]	Many	Urine*	1322	5.0	6.7	1.35
Sepkovic [95]	USA	Urine*	267	4.5	7.1	1.58

[1] Self-reported non-smokers with high cotinine levels indicative of smoking have generally been removed from the analyses presented.
[2] Except where indicated by an asterisk in the body fluid column.

sexes [19] serum cotinine levels in non-tobacco users were, respectively, 0.132, 0.381, 0.651 and 0.926 ng/ml for the same four groups. While clearly some individual non-smokers are exposed to more ETS at work than at home, these and additional data (see [113]) make it clear that the claim by OSHA (114) that the workplace is "the overwhelming exposure location" has little foundation in fact. Similarly, the approximately 2-fold higher cotinine levels associated with at home rather than at work ETS exposure cited above [19,60] suggest strongly that a theoretically derived estimate of an approximately 4-fold higher tar exposure associated with at work ETS exposure [115] (which has been used for risk estimation [116]) is likely to be misleading.

Cotinine levels and extent of ETS exposure

Quite a large number of studies have related cotinine levels in adults to questionnaire-based assessments of the extent of ETS exposure, either at home, at work or overall. Generally, a clear dose-response relationship can be seen, as, for example, in the three large representative UK studies [27,28,34] where serum cotinine in non-smokers was related to the number of cigarettes reported to be smoked per day by other household members (Table 10). Even in the highest categories, cotinine levels are typically two orders of magnitude less than those seen in smokers.

Cotinine levels in children and parental smoking

Table 11 summarizes results for a number of studies which have compared cotinine levels in children according to smoking by the parents. In some

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TABLE 10

Serum cotinine level in non-smokers[1] in relation to extent of household ETS exposure in three large representative UK studies

Study	Sex	Sample size	Cotinine level (ng/ml)[2]			
			Passive smoke exposure[3]			
			None	Light	Moderate	Heavy
Bennett [27]	M+F	3385	0.9	1.3	1.8	2.8
Calhoun [28]	M	3083	0.7	1.5	1.9	2.6
	F	3877	0.6	1.1	1.4	2.4
Prescott-Clarke [34]	M	3435	0.8	1.3	1.9	2.9
	F	4404	0.7	0.9	1.4	2.0

[1] Excluding those using nicotine replacement products, and self-reported non-smokers with cotinine levels of 20 ng/ml.
[2] Mean cotinine levels are shown: median levels are typically 30-50% lower but still show a clear dose-relationship.
[3] Based on total number reported to be smoked per day by other members of the household: light = less than 10; moderate = 10, less than 20; heavy = 20 or more.

TABLE 11

Cotinine levels in infants and non-smoking children in relation to their parents' smoking habits

Study	Country	Age[2]	Body fluid	Sample size	Cotinine level (ng/ml)[1]			
					Smoking by parents			
					Neither	Father[3] only	Mother only	Both
Bakoula [117]	Greece	1-5	Urine*	501	23.7	57.5	70.1	114.0
Bono [118]	Italy	14	Urine*	387	7.1	9.5**	12.4	16.3
Cook [119]	UK	5-7	Saliva	2721	0.33	1.17	2.20	4.05
Jarvis [120]	UK	11-16	Saliva	569	0.44	1.31	1.95	3.38
Jarvis [121]	UK	11-16	Saliva	330	1.56	2.57	3.75	6.12
Marbury [122]	USA	<2	Urine*	48	10	320	622	1650
Preston [123]	USA	2-11	Urine	175	5.9	19.7	41.8	31.1
Reese [124]	Australia	3.8	Urine*	443	31	43	39	74
Weaver [125]	USA	3.8	Urine*	79	45	80**	89	128
Willers [126]	Sweden	7.5	Urine	77	2.0	5.8	12.0	9.4
Willers [109]	Sweden	8.9	Urine*	46	1.7	6.1	8.0	13.0

[1] ng/mg creatinine where indicated by an asterisk in the body fluid column.
[2] Range, mean or median.
[3] Other household member where indicated by a double asterisk.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqhj0001

studies the father is replaced by another household member in these analyses. Though the actual levels (particularly in urine) vary markedly from study to study, the studies are reasonably consistent in showing that cotinine increases with the number of parents who smoke. The increase seen is greater if the mother smokes than if the father smokes, consistent with children spending more time in the presence of their mother than their father.

A strong association between cotinine levels in children and ETS exposure at home was also seen in a large representative US study [19]. Serum cotinine levels in children who did not use tobacco were 0.119 ng/ml at age 4–11 and 0.113 ng/ml at age 12–16 if there was no reported ETS exposure at home, but were, respectively, 1.14 ng/ml and 0.808 ng/ml if there was such exposure. Some studies (e.g. [117,127–129]) have reported a clear relationship between cotinine levels in children and the number of cigarettes smoked per day by the parents.

Cotinine levels in non-smoking children as an index of equivalent active smoking

In one study cited in Table 11 [120] the salivary cotinine level in 11–16 year old non-smoking children with a mother who smoked was estimated as 1.95 ng/ml. Noting that the active smoking of 20 cigarettes per day was associated with a salivary cotinine level of 300 ng/ml, the authors estimated that the dose of nicotine received from mother's smoking was equivalent to the active smoking of $(1.95 \times 20 \times 365)/300 = 47.45$ cigarettes per year or about 50 cigarettes per year. Similarly they estimated that the dose of nicotine received from father's smoking, where the cotinine level was 1.31 ng/ml, was equivalent to about 30 cigarettes per year, and that from both parents smoking [3.38 ng/ml] was equivalent to about 80 cigarettes per year. The authors went on to argue that "this unsolicited burden may be prolonged throughout childhood and poses a definite risk to health". There are various reasons why the conclusions are open to question. In the first place, all the salivary cotinine in children whose parents both smoke is assumed to be due to their smoking, which is inconsistent with the value of 0.44 ng/ml seen in children neither of whose parents smoke. Also, because ETS and mainstream smoke are dissimilar in their composition, properties and modes of intake. comparisons on the basis of cigarette equivalents are misleading (see also Section 3). Even ignoring these points, the conclusion that this poses a definite risk to health seems dubious. Epidemiological studies have not indicated any excess risk of disease in smokers of even 80 cigarettes per year (0.2 per day) [130] and other researchers estimating rather similar uptakes of nicotine by non-smokers have regarded them as 'negligible' [48].

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VIII. EVIDENCE OF A RELATIONSHIP OF TOBACCO SMOKE TO DISEASE

Cotinine as a marker in studies of diseases in infants and children

Cotinine has been quite widely used as a marker in studies relating ETS exposure to various diseases or conditions in infants and children. Most commonly, these studies concern aspects of respiratory morbidity, such as asthma, lung function or the presence of various symptoms [124,126,131–145]. Otitis media [146–148], sudden infant death syndrome [149,150], Down's syndrome [151], and even child development [152,153] have also been investigated using cotinine. It is beyond the scope of this paper to summarize the relevant findings. However, some points are worth noting.

First, relatively few of the studies allow one to distinguish whether cotinine or reported indices of ETS exposure correlate better with the health effects. In those that did, the evidence was not clear. Two studies [132,133] found that cotinine did not have significant predictive power over and above reported indices, one study [135] found, at least for some of the diseases studied, that it did, while another study [139] found associations with reported ETS exposure but not with cotinine.

Second, measurements of cotinine do not avoid the problems of distinguishing between potential pre- and post-natal effects on health of maternal smoking. Most smokers who smoke after pregnancy also smoke during pregnancy. Particularly in infants and young children, correlation of health indices with cotinine levels may indirectly reflect effects of smoking during pregnancy and need not necessarily indicate effects of ETS exposure.

Third, there is a theoretical possibility that metabolism of nicotine to cotinine may be affected by the presence of some diseases and also perhaps that it may be genetically linked to susceptibility to some diseases. As far as the present author is aware, these possibilities have not been studied.

Cotinine as a marker in studies of birthweight

A number of studies [23,30,38,57,59,154–158] have related birthweight of infants to cotinine levels measured in the mother during pregnancy. Some of these studies found that, after adjustment for reported number of cigarettes smoked by the mother, there was a clear association between increasing cotinine level and reduced birthweight [23,30,57,154,157,158]. One large study [30] presented a two-way table of birthweight by grouped cotinine level and by grouped reported number of cigarettes per day, which clearly showed that cotinine was a better predictor of birthweight. This may be partly because cotinine is intrinsically a better marker of relevant exposure in this context and partly because mothers' reports of amount smoked may be

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inaccurate, especially in a situation where they are likely to have been advised to give up or cut down.

Further research questions in this area include the specific time point in pregnancy at which cotinine correlates most strongly with final birthweight, and whether pregnancy itself, or complications of pregnancy, affects the metabolism of nicotine.

Cotinine as a marker in studies of diseases in adults

Studies of health effects in adults have only rarely used cotinine as a marker of the extent of smoking or of ETS exposure. Five particular studies merit comment.

The first [159] examined the relation of biochemical, physical examination, and depression assessments to self-reported cigarette consumption and serum cotinine in a sample of 743 Mexican-Americans. After controlling for sex, age and education, serum cotinine was a significant predictor of haematocrit, haemoglobin, red blood cells, white blood cells, blood lead and diastolic blood pressure. Self-reported cigarette consumption was only significant for mean corpuscular volume. The authors concluded that "serum cotinine may be a better method of quantifying risks from cigarette use in epidemiological studies." The value of cotinine must surely depend, however, on the disease in question, in particular whether its incidence is affected by nicotine exposure or by other tobacco smoke constituents and whether the disease is acute or chronic, cotinine being likely to be better correlated with recent smoking than with past smoking.

In the second study [160], conducted in Scotland, the relationship between self-reported ETS exposure, serum cotinine and evidence of respiratory or coronary disease was studied in 786 men and 1492 women who reported never having smoked tobacco and who had serum cotinine concentrations below 17.5 ng/ml. It is interesting to note that, though self-reported ETS exposure showed strong, statistically significant, dose-response relationships with various respiratory symptoms, these relationships were weak or absent for serum cotinine. The authors suggest that the presence of respiratory disease might bias self-reports of the extent of ETS exposure, in this study classified rather subjectively as 'none at all', 'a little', 'some' or 'a lot'. Using the more objective marker, cotinine, avoided such bias.

Virtually all the epidemiological studies relating ETS exposure to the incidence of lung cancer among non-smokers have relied on self- or proxy-report to obtain information on smoking habits and ETS exposure.

One exception is a large US case-control study [62] which merely used urinary cotinine levels in an attempt to exclude misclassified non-smokers. Using it as an index of ETS exposure was not attempted, presumably as cotinine measured after diagnosis would not be a reliable marker of lifetime ETS exposure. It is also true, however, that many lung cancer patients give

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up smoking at or around the time of diagnosis, and cotinine levels determined from a sample of urine taken days or weeks later would therefore still not reliably have excluded all misclassified smokers.

The other ETS/lung cancer study to use cotinine [161], involved the collection and freezing of urine samples from over 20 000 Dutch women. Over the following years, 92 of the women were identified as having contracted lung cancer and the urine samples of these women and of 305 controls were analysed for cotinine. Women identified as smokers, based on cotinine, had a significant 8-fold higher risk of lung cancer than did non-smokers, but the authors concluded that "urinary cotinine did not seem to be more predictive of lung cancer risk than self-reported cigarette consumption." Among women identified as never smokers, with no self-report of past or present smoking and no evidence of current smoking from their cotinine level, lung cancer risks were over 2-fold higher in those with a cotinine/creatinine ratio of between 9.2 and 23.4 ng/mg or between 23.4 and 100 ng/mg than in those with a ratio of less than 9.2 ng/mg. However, the differences seen were not statistically significant, the analysis being based on only 23 lung cancer cases. The study indicates the enormous effort that would be required to use cotinine as a marker of ETS exposure in a lung cancer prospective study, and obtain enough deaths for reliable inferences to be drawn.

The final study of adults using cotinine as a marker that merits particular attention, also prospective, is a study of 3270 Danish men aged 53–74 [162] who reported their previous and current tobacco habits, including chewing tobacco and snuff, and were identified as misclassified smokers based on a serum cotinine level of 100 ng/ml. Based on deaths over the next 8 years, the authors found that smokers who denied smoking had a risk of heart disease that was 4.0 times higher (95% confidence interval 1.8–9.1) than that of smokers who admitted to smoking. This finding is consistent with denial of smoking being substantially greater in smokers advised to give up by their doctors [78], smokers with a first non-fatal myocardial infarction and at increased risk of a second fatal one being particularly likely to receive such advice. While this study did not provide data on risk of disease in non-smokers in relation to cotinine level, it is of particular importance for the interpretation of the evidence on possible effects of ETS on chronic diseases, as it suggests that standard methods for adjustment for bias due to misclassification of smoking habits may underestimate bias by failing to take into account the possibility that misclassification rates may be higher in those subjects who are more at risk of disease (163).

Cotinine as a predictor of risk of chronic diseases

It can be seen from the above that there is very little evidence on which to judge how well a single cotinine measurement is likely to predict risk of subsequent death from chronic diseases such as lung cancer, heart disease or

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chronic bronchitis. By taking into account aspects of inhalation, it is possible that it might be a better predictor of risk than number of cigarettes per day in people who continue to smoke at a relevant consistent rate. However, since it is only a marker of recent smoking, it may prove a worse predictor than a lifetime smoking history. Of course, it is possible to measure cotinine at more than one time point.

Cotinine as a marker of exposure to disease risk factors other than smoking

One problem in interpreting epidemiological studies of potential effects of smoking and of ETS exposure lies in the possibility of confounding by other risk factors. While it is well known that, on average, smokers drink more alcohol and coffee than non-smokers and tend, in many countries, to be of lower social class, the full extent to which smoking, and ETS exposure, are associated with increased exposure to a variety of lifestyle risk factors is perhaps not so well realised. A recent study [164] of a representative sample of over 9000 British adults compared the distribution of 33 lifestyle risk factors generally considered to be associated with adverse health in current smokers, ex-smokers, never smokers living with a smoker and other never smokers. Of the 33 risk factors, 27 showed a significantly higher prevalence in heavy smokers than in never smokers and only two showed a significantly lower prevalence. For many risk factors, prevalence increased with amount smoked, decreased with time since smoking cessation, and was increased in never smokers living with a smoker. These results strongly suggest that some of the observed excess risk of disease in smokers and passive smokers is due to their poorer lifestyle in other respects.

These results were based on self-reports of smoking habits or ETS exposure. That using cotinine as a marker of tobacco smoke exposure does not eliminate the possibility of confounding is illustrated by some previously unpublished work I have carried out with my colleagues Jan Hamling and John Fry (fuller details of which are available on request). This was based on data from two UK studies. The first (Health and Lifestyle Survey follow up=HALS2) was based on reinterviews of the same population used for the study referred to above [164], in which data on saliva cotinine, self-reported smoking habits and ETS exposure, and 27 of the 33 risk factors originally studied were available on 3668 adults. The second (Health Survey of England 1993=HSE93) was a survey of 4813 adults, in which data on serum cotinine, self-reported smoking habits and ETS exposure, and many identical or similar risk factors were available.

Relationship of cotinine levels in never smokers to other risk factors (HALS2 and HSE93 studies)

Table 12 shows how, in never smokers, age and sex adjusted prevalence of many of the risk factors varies markedly by cotinine level. The table shows

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TABLE 12

Prevalence of lifestyle health risk factors in never smokers[a] classified by cotinine level

	Saliva cotinine level (ng/ml)[b]						
	0.0-0.5	0.6-1.0	1.1-2.0	2.1-5.0	5.1-30.0	HALS2 Trend	HSE93 Trend
Risk factor[c]	%[d]	%[d]	%[d]	%[d]	%[d]	p[e]	p[e]
Father dead	61.1	59.8	64.6	61.4	67.7	NS	NS
Mother dead	44.6	41.5	45.7	46.7	59.5	+	+
No educational qualifications	28.2	30.1	39.0	38.7	56.7	+++	+++
Separated, divorced or widowed	12.5	15.0	18.4	18.5	17.5	+	NS
Not in paid employment	38.2	32.9	34.3	34.1	40.2	NS	NS
Low social class	37.3	41.6	53.3	54.1	63.1	+++	+++
High alcohol consumption	18.2	23.5	23.2	35.7	29.3	++	+++
High bread consumption	45.5	42.0	47.0	49.6	52.2	+	NS
Low fruit consumption	26.3	22.8	31.8	30.2	39.5	++	+++
Low vegetable consumption	50.1	48.1	51.0	49.4	56.6	NS	
Low vegetable/salad consumption[f]	27.6	27.1	27.8	32.9	39.7		+++
Low salad consumption	49.3	46.0	46.6	49.2	52.5	NS	
High sweet food consumption	59.8	58.9	55.0	49.7	48.1	− −	−
High salt consumption in food[f]	37.4	39.0	40.3	38.3	47.4		++
No use of low fat/PU spread	21.7	20.5	20.1	27.7	38.6	++	NS
High body mass index	51.1	55.9	58.1	62.7	64.6	+++	++
Underweight	2.3	1.0	1.1	1.3	2.8	NS	NS
'Risky' occupation	21.9	21.2	26.5	33.2	44.3	+++	
Low income	45.2	44.0	49.2	53.8	57.6	+++	
Do nothing to keep healthy	28.3	29.6	28.6	35.8	43.8	++	
Low activity[f]	13.1	15.8	14.6	12.9	15.3		NS
Little sleep	37.7	38.5	36.5	46.5	42.9	NS	
Long time before first meal	14.7	17.4	19.0	21.1	15.9	++	
High fried food consumption	15.3	20.1	20.5	24.6	29.8	+++	
Low breakfast cereal consumption	25.1	28.4	30.3	29.1	39.0	+++	
Sugar in tea or coffee	35.6	35.5	39.8	36.8	51.7	+	
High tea consumption	18.5	21.0	17.8	20.0	22.2	NS	
High coffee consumption	7.2	5.3	8.5	6.7	7.1	NS	
High neuroticism	39.8	44.6	54.0	48.2	42.2	(+)	
High extroversion	32.0	42.5	43.1	49.2	56.1	+++	
Low control at work[f]	17.7	20.4	24.1	28.8	32.2		+++
High pace index[f]	31.6	39.6	37.1	33.7	33.8		NS
Number of subjects (HALS2)	511	278	241	221	93		
Number of subjects (HSE93)[g]	353	485	390	263	194		

[a] Excluding never smokers with no data on social class or living with a smoker and with either saliva cotinine above 30 ng/ml (HALS2) or with serum cotinine above 20 ng/ml (HSE93).
[b] Where data for the risk factor are available from HALS2, prevalences shown are for HALS2 data. Otherwise prevalences shown are for HSE93 with categories, based on serum cotinine, of 0.0–0.2, 0.3–0.5, 0.6–1.0, 1.1–2.0 and 2.1–20.0 ng/ml.
[c] A detailed definition of the risk factors and the high/low cut offs used is available from the author.
[d] Percentages are adjusted for sex and age (<30, 30–39, 40–49, 50–59, 60–69, 70+) to the overall population of never smokers considered.
[e] Significance codes: +++, −−− $p<0.001$, ++, −− $p<0.01$, +, − $p<0.05$, (+), (-) $p<0.1$. NS not significant $p \geq 0.1$. Plus signs indicate increasing trend, minus signs decreasing trend.
[f] Only data for HSE93 available.
[g] See note b for categories used.

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results of trend tests for both surveys, where available, but only shows prevalence by cotinine level for one of the surveys (HALS2 if available) as patterns were generally found to be quite similar in the two surveys. Never smokers with cotinine levels consistent with active smoking are excluded from the analyses.

The results clearly show that though a high cotinine level in a never smoker is a marker of increased ETS exposure, it is also a marker of many other things. Thus, it is clearly associated with poorer education, lower income, lower social class, with working in a job with increased potential exposure to carcinogens, with higher alcohol consumption, with higher extroversion scores, with a higher body mass index and with higher fried food consumption, and with lower consumption of fruit, breakfast cereal and sweet foods. Additional analysis (data not shown) showed that prevalence of risk factors was generally more strongly associated with cotinine level than with the index "living with a smoker".

Relationship of cotinine levels in cigarette smokers to other risk factors (HALS2 and HSE93 studies)

Table 13 compares prevalences in never smokers and in current cigarette smokers classified by cotinine level. Based on trends including the never smoking group, which to some extent reflect the current smoker/never smoker difference, the table shows that increased smoking (as quantified by cotinine) is associated with a highly significant ($p<0.001$) increase in prevalence of virtually all the risk factors studied, the notable exceptions being that increased smoking is associated with a lower body mass index and with lower sweet food consumption.

The significance of trends in prevalence by cotinine level in analyses restricted to current cigarette smokers was also calculated, though is not shown in Table 13. For many of the risk factors, including no educational qualifications, low social class, low fruit consumption, low salad consumption, high salt consumption, no use of low fat/polyunsaturated spread, 'risky' occupation, do nothing to keep healthy, long time before first meal, high fried food consumption, low breakfast cereal consumption, taking sugar in tea or coffee, high tea consumption, high coffee consumption, the trend seen remained significant at least at $p<0.05$. However for others they were not apparent, indicating the major difference in prevalence is between current smokers and never smokers. Interestingly, however, in both studies, though frequency of high alcohol consumption was clearly commoner in current than in never smokers, it was (in both studies) significantly negatively related to cotinine level in current smokers.

Further analyses (data not shown) showed that while, for some risk factors, prevalence, in smokers, was more strongly associated with cotinine level, for others it was more strongly associated with number of cigarettes

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TABLE 13

Prevalence of lifestyle health risk factors in current cigarette smokers classified by cotinine level and in never smokers[a]

	Never smoked[a]	Current cigarette smoker					
		Saliva cotinine level (ng/ml)[b]					
		<200	200.1–350	350.1–450	>450	HALS2 Trend[c]	HSE93 Trend[c]
Risk factor[d]	%[e]	%[e]	%[e]	%[e]	%[e]	p[f]	p[f]
Father dead	60.6	60.1	63.7	65.9	62.5	(+)	+
Mother dead	43.9	41.2	48.6	49.6	48.3	++	NS
No educational qualifications	32.7	45.0	52.2	58.5	54.5	+++	+++
Separated, divorced or widowed	14.1	19.7	19.7	23.9	22.2	+++	+++
Not in paid employment	34.5	41.4	41.5	40.5	43.6	+++	+++
Low social class	45.3	61.0	65.7	65.5	72.9	+++	+++
High alcohol consumption	24.4	43.7	42.5	35.4	30.7	+++	+++
High bread consumption	47.6	46.9	53.5	50.6	52.1	+++	NS
Low fruit consumption	28.7	49.6	51.5	60.1	59.8	+++	+++
Low vegetable consumption	51.5	51.6	51.2	55.1	58.3	+	
Low vegetable/salad consumption[g]	30.5	39.4	39.1	41.2	43.4		+++
Low salad consumption	48.9	52.6	61.4	66.0	71.5	+++	
High sweet food consumption	55.6	38.6	36.7	33.2	38.4	- - -	- - -
High salt consumption in food[g]	40.1	55.0	51.4	61.0	56.0		+++
No use of low fat/PU spread	22.4	34.0	32.5	35.2	47.3	+++	+++
High body mass index	55.3	48.5	56.3	47.3	48.1	- - -	- - -
Underweight	1.9	2.7	5.1	4.7	5.0	+++	+++
'Risky' occupation	26.3	31.7	38.9	36.6	46.6	+++	
Low income	46.1	61.4	58.1	60.9	+++		
Do nothing to keep healthy	30.9	39.2	40.7	39.8	48.1	+++	
Low activity[g]	13.8	10.9	20.3	15.0	11.0	+++	+++
Little sleep	38.6	46.6	46.4	47.6	47.7	+++	
Long time before first meal	17.4	23.9	40.1	48.5	52.3	+++	
High fried food consumption	20.1	30.5	33.6	33.2	40.4	+++	
Low breakfast cereal consumption	28.8	43.1	50.4	54.9	54.9	+++	
Sugar in tea or coffee	37.8	50.9	53.7	56.4	66.0	+++	
High tea consumption	19.2	28.7	39.6	44.4	50.5	+++	
High coffee consumption	6.6	17.3	20.2	27.5	27.7	+++	
High neuroticism	43.1	55.9	53.1	47.7	49.0	+++	
High extroversion	40.6	56.3	53.3	56.2	53.1	+++	
Low control at work[g]	23.0	14.3	28.5	28.4	23.5		++
High pace index[g]	35.6	39.6	29.3	36.2	23.3		NS
Number of subjects (HALS2)	1344	229	340	235	259		
Number of subjects (HSE93)[h]	1685	278	414	317	262		

[a] Excluding never smokers with no data on social class or living with a smoker and with either saliva cotinine above 30 ng/ml (HALS2) or with serum cotinine above 20 ng/ml (HSE93).
[b] Where data for the risk factor are available from HALS2, prevalences shown are for HALS2 data. Otherwise prevalences shown are for HSE93 with categories, based on serum cotinine, of <100, 100.1–250, 250.1–350, >350 ng/ml.
[c] Trend including never smokers.
[d] A detailed definition of the risk factors and the high/low cut offs used is available from the author.
[e] Percentages are adjusted for sex and age (<30, 30–39, 40–49, 50–59 and 60–69, 70+ for males or 60+ for females) to the overall population considered.
[f] Significance codes: +++, ---$p<0.001$, ++, --$p<0.01$, +, -$p<0.05$, (+), (-)$p<0.1$, NS not significant $p \geq 0.1$. Plus signs indicate increasing trend, minus signs decreasing trend.
[g] Only data for HSE93 available.
[h] See note b for categories used.

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Source: https://www.industrydocuments.ucsf.edu/docs/hqhj0001

smoked. This may reflect the fact that, in smokers, cotinine is an indicator not only of how many cigarettes are smoked, but also of how much cotinine is obtained per cigarette. The depth to which people inhale cigarettes and the extent to which they metabolize nicotine to cotinine may vary according to various factors.

Effect of alcohol consumption on cotinine levels (HALS2 and HSE93 studies)

The data relating to alcohol consumption from the two surveys are particularly interesting in this respect. While the frequency of high alcohol consumption in smokers clearly increases with increasing amount smoked [164], it clearly decreases with increasing cotinine level (see Table 13). In other words, increasing alcohol consumption is associated with decreased cotinine per cigarette smoked. This may be due to heavy drinking being associated with reduced inhalation, but other possible explanations include alcohol having an effect on the method used to determine cotinine or on the metabolism of nicotine. An effect of alcohol on nicotine metabolism is supported by the results of a pharmacokinetic study in rats pretreated with ethanol [165]. It is interesting to note that if alcohol affects nicotine metabolism in humans, adjustment for alcohol consumption may be necessary in studies using cotinine as an index of exposure to active smoking or ETS.

Effect of body mass index on cotinine levels in smokers (HALS2 and HSE93 studies)

Body mass index was another risk factor found in our analyses to be positively related to number of cigarettes smoked and negatively related to cotinine. In a previous report of a similar finding [91] nicotine metabolism, energy intake and measurement issues were considered as possible explanations. These findings are consistent with those of another study [166] which reported increased salivary cotinine concentrations in smokers during rapid weight loss following participation in a protein-sparing modified fast.

Relationship of cotinine to factors other than smoking – some evidence from studies other than HALS2 and HSE93

A large number of other studies have reported data relating cotinine levels in adults or children to factors other than smoking. The evidence seems to be quite consistent that increased cotinine levels are associated with low social class or poor education [17,119,167–170], Black race [19,57,152,168,170–173], household crowding or small room size [19,128,169] and alcohol and drug consumption [21]. Cotinine levels in male

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and female smokers of the same number of cigarettes appear, however, to be similar (see Table 6). Pregnancy was not associated with decreased cotinine levels in women when measured at the time of diagnosis [174].

Consistent with evidence from earlier studies (175,176), a recent study (177) reported levels of urinary cotinine in the infants of nursing mothers who smoked were markedly higher if they were breast-fed than if they were bottle-fed. The authors suggested breast-feeding, rather than direct inhalation of ETS, is their primary determinant of cotinine.

IX. OTHER ISSUES

Correlation between multiple cotinine measurements taken at different time points

Cotinine levels are determined by smoking and ETS exposure in the last few days, yet are used as markers in studies of diseases which may reflect exposure to tobacco smoke constituents over months or years. If cotinine levels vary markedly over time, due to variations in exposure, metabolism, etc., a single cotinine measurement may substantially underestimate any true relationship of smoking or ETS exposure with disease. If, on the other hand, cotinine levels are quite consistent over time, a single measurement may be more relevant.

A few studies have investigated the consistency of cotinine measurements taken in the same subjects at different points of time. In one such study, of 169 girls aged 11–16 in which salivary cotinine was measured in 1985 and again in 1986 [121], there was a correlation of 0.75 between the two measurements. Another study, of 111 children aged 5–7 [135], reported a correlation of 0.81 between measurements taken at 6-month intervals. In a third study, in which plasma cotinine in 148 smokers was measured on two occasions separated in time by 14 weeks [31], the correlation was 0.81.

One paper [83], reported results from two studies of cigarette smokers, one of 200 men providing blood on 3 occasions, the other of 51 men and 52 women providing blood on 6 occasions. In both studies, standard deviations of blood cotinine within smokers, of about 63 ng/ml, were 2 to 3 times less than standard deviations between smokers.

In a study of 20 US non-smokers measurements of urinary cotinine on 10 days over a 10 week period [178] the highest values recorded for a subject were typically about twice as high as their mean value.

In a recent study, an attempt was made to collect urine for cotinine determination every two months from birth to two years of age in 99 infants [179]. When individual values for an infant were compared to the average of the multiple measurements, about a third were in error by more than 100 ng/mg. Classifying infants as having low or high exposure depending on whether or not 50% of their readings were less than 32 ng/mg, 84% were correctly classified by their individual values.

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In a detailed study of ETS exposure in 91 asthmatic children [180], the two month test-retest correlation coefficient was in the range 0.60 to 0.88 for nearly all the indices studied, including parental smoking, cigarette butts and ETS as determined by an air monitor. However, for urinary cotinine, it was only 0.04, and the authors considered it unreliable.

In a further study [181], levels of salivary cotinine in two smokers were recorded from samples collected daily over a one month period. The maximum values coincided with weekends or holidays when the individual's smoking behaviour was not recorded.

Generally, though not always, these results suggest that a single cotinine determination may be useful in studies of tobacco smoke exposure and disease. However, multiple measurements will clearly add power to detect any true associations of average cotinine level with disease.

Correlation between cotinine measurements taken in different body fluids

In some studies, cotinine has been measured in more than one body fluid. Correlations have, for example, been reported between levels in saliva and serum, $r=0.84$ [67], urine and blood, $r=0.72$ [182], and saliva and urine, $r=0.92$ [183]. Although these correlations are relatively high, they are far from perfect, and suggest that the value of cotinine, as a marker of tobacco smoke exposure, may vary in relation to the body fluid in which it is measured.

Correlation between cotinine levels and air nicotine levels

A number of studies of children and non-smoking adults have reported the correlation between cotinine levels, as measured in urine or saliva, and nicotine levels, as measured from air samples taken by personal monitors or passive monitors placed in the room [77,122,178,184–192]. While positive correlations are always reported, they are far from perfect, never exceeding more than about 0.8 and typically averaging around 0.5.

The lack of perfect correlation will partly be due to errors in determining cotinine levels and air nicotine levels, and partly because the former, but not the latter, will be affected by inter-individual differences in inhalation and nicotine metabolism.

Metabolism of nicotine and half-life of cotinine

In a study in which six healthy volunteers were infused over four days with doses of nicotine [193] varying up to 480 μg/kg/day, cotinine concentrations in plasma were found to be linearly and directly related to nicotine intake. The mean regression line was cotinine concentration (μg/l) = 0.783 × nicotine (μg/kg/day).

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The half-life of cotinine has been reported variously as 12 h [194], 17 h [18], 16–19 h [195], 19 h [196], 29 h [197] and 55 h [184]. For a half-life of 18 h, an average smoker would have to have stopped smoking for about four days before his cotinine level declined to that typical of an ETS-exposed non-smoker and for about a week before his cotinine level became undetectable using modern analytical methods.

In one study in which plasma, saliva and urinary cotinine concentrations were determined in eight smokers who stopped smoking [198], plasma and urinary levels dropped markedly over a five-and-a-half day period, by a factor of 40 or more. In contrast, salivary cotinine levels only dropped by a factor of two over this period. However, a subsequent study failed to replicate this finding, reporting no tendency for the half-life in saliva to be longer than in plasma or urine [195].

The published evidence appears to be somewhat conflicting regarding whether smokers metabolize nicotine to cotinine differently from non-smokers. While one study reported nicotine clearance was significantly slower in smokers [199], some other studies [58,200,201] have reported a longer half-life in non-smokers. However, two of these studies [200,201] used radiolabelled racemic nicotine, which is a mixture of two forms of nicotine, only one of which is present in tobacco. Furthermore, other studies [18,195.202.203] have reported that the pharmacokinetics of nicotine and/or cotinine are similar in smokers and non-smokers.

One study [173] compared nicotine metabolism and intake in black and white smokers and explained the higher levels of cotinine per cigarette smoked by blacks in terms of their slower clearance of cotinine and higher intake of nicotine per cigarette.

A possible limitation of cotinine as a marker of nicotine exposure is highlighted by the report [204] of a woman who was found to have the expected plasma levels of nicotine but unusually low plasma levels of cotinine. both when smoking cigarettes and while receiving transdermal nicotine. The subject converted only 9% of nicotine to cotinine, compared to 72% for control subjects. The frequency of such deficient C-oxidation of nicotine to cotinine is not known, but it may help to explain why some smokers have low cotinine levels [17,19], it being generally regarded as implausible that adults would claim to smoke when they did not in fact do so.

Storage of samples

In one study [205]. the ratio of cotinine as determined by: (a) radio-immunoassay; and (b) gas chromatography, was measured both in fresh serum samples and in samples stored for more than four years. The ratios in the two sets of samples were similar for serum from smokers but were much less consistent in the stored samples for serum from non-smokers. The

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authors suggested that aging may affect the determination when dealing with low levels of cotinine.

In another study [206], plasma left at room temperature for 12 days had cotinine concentrations which correlated highly [r=0.99] with those in duplicate samples frozen immediately. There was no evidence of a shift in values or an increase in variability due to delayed freezing.

In a third study [207], two saliva specimens were taken simultaneously from 10 smokers and 10 non-smokers. One was frozen immediately and the other sent through the local US mail and then subjected to additional physical agitation and heat before being frozen. No cotinine was detectable in any of the non-smokers' specimens, and there was good agreement [r=0.88] between the two specimens for the smokers. The authors concluded that the mailing of saliva specimens for cotinine analysis was practical. Similar conclusions were reached in another study [208], which compared saliva samples, one of which was immediately stored on ice and transported to the laboratory, the other being mailed immediately by the subject to the laboratory.

In a further study [209], cotinine was measured in 1988 in urine collected in 1976–77, at a time when data on smoking status were also collected. Cotinine measurements showed a clear separation of smokers and non-smokers (specificity 95%, sensitivity 100%), suggesting that cotinine retained its ability to discriminate, even after 10 years of storage.

A conclusion from these data seems to be that one can satisfactorily use stored samples for cotinine analysis. However a study [210] in which urine, taken from smokers, was stored for 30 days at temperatures of 5, 25, 40, 50 or 60°C shows that this conclusion does not hold true for storage at high temperature. While cotinine levels remained relatively constant at 5°C they increased with time at higher temperatures, doubling over the 30 day period at 60°C. This is presumably due to release of cotinine from its glucuronide conjugate.

X. SOME GENERAL CONCLUSIONS

Cotinine is the main metabolite of nicotine, the principal alkaloid of the tobacco plant. Since 1966, when a reasonably reliable chemical method for its determination was first described [211], techniques for its determination in a wide range of body fluids have been developed (see Chapter 8). As described in the previous sections of this paper, cotinine determinations have been made in many human populations for many purposes.

There are a number of advantages in using cotinine as a marker of tobacco smoke exposure, which are summarized below.

Objectivity. Most epidemiological studies relating smoking or ETS exposure to the incidence of various diseases have relied upon data reported by the

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subject or another family member. Such data are unreliable [47] and errors are clearly not random. The well documented tendency [78–80] for patients advised to give up smoking to be particularly likely to deny later that they have failed to do so, is one illustration of this. Use of an objective marker of exposure, such as cotinine, avoids such recall bias, and indeed in one study [162] was able to demonstrate a high subsequent risk of coronary death in misclassified smokers. The results of a Scottish study [160] which demonstrated an association of respiratory symptoms with self-reported ETS exposure but not with cotinine are consistent with use of an objective marker avoiding the bias which arises because subjects with symptoms tend to perceive their ETS exposure as more severe.

Sensitivity. Cotinine levels in humans can be detected over more than a thousand-fold range. Not only can they clearly distinguish smokers from non-smokers (providing care is taken to exclude other sources of nicotine such as chewing tobacco, nicotine gum and nicotine patches), but, at the lower end of the detectable range, can discriminate non-smokers with high, medium or low ETS exposure.

Practical uniqueness to tobacco. It has been argued [45] that an appropriate marker of exposure to tobacco smoke must be unique to tobacco. From an epidemiologist's viewpoint, such an argument is not correct; the ideal marker is one that measures exposure to the actual agent that causes the disease under consideration, whether or not the agent is present only in tobacco smoke. However, from the point of view of distinguishing smokers from non-smokers, or of quantifying relative exposure of different smokers or relative ETS exposure of different non-smokers, use of a marker that is not affected in a major way by non-tobacco sources is an advantage. As discussed in section 2, nicotine is present in diet [9–11], but not at levels that are likely to mimic more than moderate ETS exposure at most [15–20].

Index of inhalation. While some epidemiological studies do include a question concerning inhalation by smokers, these data are regarded as rather soft, as the concept is difficult to quantify and smokers have little detailed knowledge of how far into their lungs their smoke goes. An attractive feature of cotinine, as a marker of tobacco smoke exposure, is that it attempts to measure uptake rather than available dose, as would be measured by number of cigarettes smoked per day. In theory, two smokers of 20 cigarettes per day could smoke very differently, not only in terms of inhalation but also in terms of puffing characteristics, so that they have very different relevant exposures to the target tissue.

There are also a number of limitations in using cotinine.

Marker only of recent exposure. Though cotinine has a much longer half-life than nicotine, and is therefore not nearly so dependent on when the last cigarette was smoked or when the last heavy ETS exposure occurred, the fact

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that its half-life in serum or urine is only about a day or so [18,194–197] means that it is only a marker of quite recent exposure. A cotinine measurement taken on a Friday will hardly reflect exposures on the previous weekend and may differ from that taken on a Monday, especially if the subject smokes more heavily at home than at work or goes out to smoky bars on a Friday or Saturday night. Though measurement of nicotine in hair may allow detection of exposure over a period of weeks or months [8], nicotine-based markers can seemingly not be used as reliable markers of lifetime exposure, unless they are taken at regular intervals.

Marker only of nicotine exposure. If comparisons are made between non-smokers with no, light or heavy ETS exposure, the relative increases in marker levels associated with heavy and light exposure would be expected to be similar regardless of the marker used. In this context, the fact that cotinine is only strictly an index of exposure to nicotine, need not matter much, as a doubling of the cotinine increase associated with heavy rather than light exposure might reasonably be taken to indicate that exposure to all constituents of ETS is approximately doubled. A similar argument might be applied to increases in markers in smokers associated with different numbers of cigarettes smoked, though perhaps less confidently as for some chemicals saturation might occur, with a doubling of cigarettes smoked not associated with a doubling of marker levels.

However, the fact that nicotine is predominantly in the particulate phase in mainstream smoke and in the vapour phase in ETS, and the fact that inhalation of tobacco smoke by smokers is very different from normal breathing by non-smokers implies that the exposure of smokers compared to non-smokers as determined by a nicotine-based marker, such as cotinine, may be very different from markers based on other tobacco smoke constituents. Certainly cotinine cannot be used to determine relative retention of particles in smokers and non-smokers.

Analytical error at low levels. The inaccuracy of cotinine determinations is proportionately much greater at low levels than at high levels. Indeed, even with the most sensitive methods, some non-smokers have levels below the detectable limit, and for less sensitive methods, such as some radio-immunoassay techniques, there are problems due to non-specificity [42].

Index of metabolism. Cotinine is only one metabolite of nicotine and the speed and extent to which cotinine is metabolised to nicotine may vary for a number of reasons. Inasmuch as the intent of using cotinine is to measure exposure to tobacco smoke, the report of a women who metabolised very little nicotine to cotinine [204] may be important, especially if she is shown not to be a one-off case. The evidence regarding factors, genetic or environmental, which may affect metabolism to cotinine is very limited, and more research is needed. If, for example, it were shown that certain diseases affected

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metabolism, there would be obvious problems in using cotinine as a marker of tobacco smoke exposure in studies investigating the relationship of smoking as ETS exposure to these diseases.

Marker of exposure to other risk factors. Just as self-reported smoking and ETS exposure are correlated with a wide range of other disease factors [164], the same is true for cotinine (Tables 12 and 13). This indicates that while use of cotinine as a marker in studies attempting to relate tobacco smoke exposure to disease can avoid recall bias, it does not avoid potential problems of confounding. Indeed, if some of the associations between cotinine and other risk factors are due to effects of the risk factors on metabolism of nicotine to cotinine, the potential for bias due to uncontrolled confounding is increased. In this context, the observation that cotinine per cigarette smoked decreases with increasing alcohol consumption and with increasing body mass index is of relevance, as it raises the possibility that alcohol and body mass index may affect metabolism.

Bearing in mind these advantages and limitations and the evidence summarized in this chapter, it is possible to come to a conclusion concerning the usefulness of cotinine as a marker in the various contexts in which it has been used.

Provided that the possibility of other sources of nicotine such as chewing tobacco, gum and patches can be excluded, it is clear that cotinine is very useful for identifying true current smokers. It is perhaps slightly less useful for identifying true current non-smokers. This is because some smokers smoke or inhale so little that their cotinine levels become indistinguishable from those of non-smokers with high ETS exposure, and because smokers vary in the rate at which they metabolise nicotine to cotinine, with some metabolising very little.

Cotinine is also of value in categorising non-smokers in terms of light, moderate or heavy ETS exposure, though the possibility cannot be excluded that dietary nicotine may affect the categorisation in a few subjects.

Cotinine is also of value in demonstrating that, in most populations at least, the home is on average a greater source of ETS exposure than is the workplace (see Section 7).

Cotinine may also be of value in categorising smokers in terms of extent of exposure to tobacco and hence in terms of relative propensity to smoking-associated diseases. Whether cotinine is of more value than a reported smoking history is likely to depend on a number of factors, including number of cotinine measurements taken, relevance of nicotine to the disease in question, relevance of inhalation to the disease in question, and whether the disease is an acute or chronic one.

Cotinine is also of some value in indicating that smokers switching to a brand of cigarettes with a lower yield of nicotine as determined by a smoking machine partly, but by no means completely 'compensate' for the reduced

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yield by increasing their inhalation. However, it does not directly demonstrate that the compensation is specifically for nicotine.

While cotinine can discriminate between levels of ETS exposure in non-smokers, it is unreliable in estimating the actual (*Z*-) ratio of exposure in non-smokers married to a smoker and married to a non-smoker, for reasons discussed in more detail in Section 7, which include sampling error, analytical error at low cotinine levels and the contribution of diet.

Cotinine is clearly extremely unreliable for estimating the relative tobacco smoke exposure of smokers and non-smokers (and for expressing ETS exposure in terms of cigarette equivalents [120]). The problems include differences between inhalation by smokers and normal breathing by non-smokers, differences in the phase distribution of nicotine in mainstream smoke and ETS, analytical error at the low levels of cotinine seen in non-smokers and the possibility that smoking itself affects metabolism of nicotine. The relative nicotine exposure of smokers and non-smokers, even if it could be accurately measured by cotinine, may differ markedly from the relative exposure to other constituents.

Cotinine is also somewhat unreliable when trying to demonstrate that heavy smokers inhale less [97], as discussed in Section 5, and in drawing inferences regarding the lack of effect of nicotine on heart disease based on the similarity of cotinine levels in pipe and cigarette smokers [36], as discussed in Section 6.

Overall, the general conclusion is that cotinine is a marker of tobacco smoke exposure which is very useful in some contexts, but far less useful in others. Care should therefore be taken to take its limitations into account.

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